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16. ABSTRACT

A bioassay for rhizosphere-applied phytotoxicants was developed and evaluated with a broad range of chemicals. Test substances were applied to the rhizoshpere of whole, intact bush bean plants (Phaseolus vulgaris L. cv. Bush Blue Lake 290) grown in a solid support medium and the resultant ethylene production was measured to detect the presence of phytotoxic materials. The beans were encapsulated in plastic bags for 2hr following treatment and then incubated for 24 hr in the dark. Ethylene and ethane accumulating within the bags were quantified via gas-solid chromatography. The application of various concentration of inorganic and organic chemicals induced various responses. No single equation adequately described the dose-response curves; therefore, a critical value (a statistically significant increase in stress ethylene production) was computed for each test substance. A phytotoxic-response threshold for each test substance was defined as the test substance concentration that caused ethylene production to exceed its respective critical-value concentration. Based on threshold concentrations determined by analysis of stress ethylene production, the relative phytotoxicity rankings of the inorganic test substances were: $CdCl_2 > CuCl_2 > Pb(C_2H_3O_2)_2 > LiCl$, while those of the organic test substances were 2,4-D esters < paraquat dicloride < 1-butanol < 2-propanone < 2-propanol. Both stress ethylene and ethane production were nonresponsive to hydrogen-ion concentration measured before application) over a broad range. Significant ethane production was detected at pH2 and stress ethylene was produced at pH1.

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STRESS ETHYLENE: A BIOASSAY FOR RHIZOSPHERE-APPLIED PHYTOTOXICANTS

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Abstract. A bioassay for rhizosphere-applied phytotoxicants was developed and evaluated with a broad range of chemicals. Test substances were applied to the rhizosphere of whole, intact bush bean plants (*Phaseolus vulgaris* L. cv. Bush Blue Lake 290) grown in a solid support medium and the resultant ethylene production was measured to detect the presence of phytotoxic materials. The beans were encapsulated in plastic bags for 2 hr following treatment and then incubated for 24 hr in the dark. Ethylene and ethane accumulating within the bags were quantified via gas-solid chromatography. The application of various concentrations of inorganic and organic chemicals induced various responses. No single equation adequately described the dose-response curves; therefore, a critical value (a statistically significant increase in stress ethylene) was computed for each test substance. A phytotoxic-response threshold for each test substance was defined as the test-substance concentration that caused ethylene production to exceed its respective critical-value concentration. Based on threshold concentrations determined by analysis of stress ethylene production, the relative phytotoxicity rankings of the inorganic test substances were: $CdCl_2 > CuCl_2 > Pb(C_2H_3O_2)_2 > LiCl$, while those of the organic test substances were 2,4-D esters > paraquat dichloride > 1-butanol > 2-propanone > 2-propanol. Both stress ethylene and ethane production were nonresponsive to hydrogen-ion concentration (measured before application) over a broad pH range. However, significant ethane production was detected at pH 2 and stress ethylene was produced at pH 1. The measurement of stress-induced ethylene provides a rapid and simple means to determine the relative phytoxicity of rhizosphere-applied substances.

1 Introduction

Ethylene produced by biotic and abiotic stresses in excess of hormonal amounts is termed stress ethylene (Abeles, 1973). Stress ethylene is produced by intact, physiologically perturbed cells, while ethane is associated with cellular decompartmentalization (Elstner and Konze, 1976). Within limits, there is a proportional relationship between the degree of stress that a plant receives and the production of stress-induced ethylene (Tingey *et al.*, 1976; Tingey, 1980). Both hormonal and stress ethylene are produced by a methionine-dependent pathway (Lieberman, 1979; Hogsett *et al.*, 1980; Yu and Yang, 1980).

Stress ethylene production is induced by air pollutants (ozone, chlorine and sulfur dioxide), aqueous solutions of metal salts (e.g., $CuCl_2$, $FeCl_3$, $HgCl_2$ and $NiCl_2$), herbicides and defoliants applied to foliage and picloram and $CdCl_2$ solutions applied to roots (Baur and Morgan, 1969; Ben-Yehoshua and Biggs, 1970; Nakagaki *et al.*, 1970; Webb *et al.*, 1975; Tingey, 1980). Measurement of stress-induced ethylene has been proposed as a bioassay for bacterial toxins (Shain and Wheeler, 1975), identifying defoliants (Webb *et al.*, 1975) and assessing the phytotoxicity of various chemicals on plant foliage (Tingey, 1980; Thompson *et al.*, 1981).

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A bioassay for rhizosphere-applied phytotoxicants was, therefore, developed and evaluated with a broad range of inorganic and organic chemicals. The test substances were applied to the rhizosphere of whole, intact plants grown in a solid support medium. The resultant ethylene production was measured to detect the presence of phytotoxic substances. Subsequently, the test substances were ranked according to their relative phytotoxicity.

2. Materials and Methods

Beans (*Phaseolus vulgaris* L. cv. Bush Blue Lake 290) were germinated and grown in 10-cm diameter pots containing vermiculite (# 2 grind, horticultural grade) and thinned to one seedling per pot 7 to 8 days after seeding. The plants were grown in a greenhouse at maximum day temperatures of 30 ± 3 °C and minimum night temperatures of 16 ± 2 °C. The plants were exposed to light 16 hr per day by supplementing sunlight with high intensity discharge sodium vapor lamps to yield an intensity of 9.2–10.5 megajoule $m^{-2} d^{-1}$ (280–2200 nm) at plant height. The plants were irrigated with an excess of modified Hoagland's solution and tap water for 5 and 2 days per week, respectively. A measure of plant development (plastochron index) was used to insure standardized plant growth (Maksymowych, 1973). Plant populations had mean plastochron indices between 1.0 and 2.1 (20 mm control leaf length) 1 day prior to treatment.

Test solutions were prepared from reagent grade chemicals except for commercial formulations of propylene glycol butyl ether esters of 2,4-dichlorophenoxyacetic acid (44.9% 2,4-D acid equivalent) and paraquat dichloride (29.1%). Test substances, except 1-butanol, were soluble or miscible in water at the concentrations tested. The 1-butanol solutions were swirled vigorously prior to their application to individual plants to minimize phase separation. Test solutions, which replaced the nutrient solution application, were applied to the rhizosphere by slowly distributing 50 ml of the desired concentration uniformly across the vermiculite surface while preventing solution contact with the stem or foliage. The application volume was chosen because it wetted the vermiculite and usually produced a few milliliters of leachate. Leachates from the plants receiving applications of test substances were contained on large, shallow trays. Water, prepared by a reverse osmosis technique and having a resistivity of 0.9 megaohms cm^{-1}, was used as the control solution. All plants received 3 to 4 hr of light before test substance application.

After the test solution was applied, the plants remained in the greennouse generally for 2 hr. They were then encapsulated in plastic bags supported by wire frames (Figure 1) to minimize foliar contact with the bag and to provide a relatively constant volume (Tingey, 1980). For comparative purposes, some plants were encapsulated immediately following the application of test substances. Some plants receiving applications of $Na_2B_4O_7$ were encapsulated 5 or 10 hr after treatment. All plants were incubated at 26 ± 1 °C in the dark for 24 hr.

The ethylene and ethane were sampled fom the bag immediately after incubation was completed with a 1-ml gastight syringe, separated on a Porapak N column

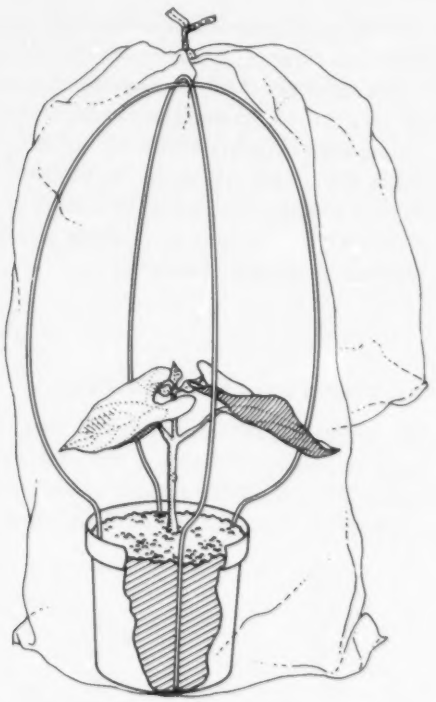

Fig. 1. Encapsulation system for stress ethylene showing cutaway, side view. Bag dimensions were 25.4 × 81.3 cm. Volume of encapsulation system was approximately 4 l.

TABLE I

Relationship between the means and standard deviations for various ethylene concentrations[a]

Test substance and concentration (M)	Mean ethylene concentration ($\mu l\ l^{-1}$)	Standard deviation	Geometric mean ethylene concentration ($\mu l\ l^{-1}$)	Standard geometric deviation
$CdCl_2$				
0	34.8	16.0	32.3	1.47
5×10^{-4}	299.5	118.1	281.9	1.43
1×10^{-3}	443.0	117.1	426.1	1.38
5×10^{-3}	784.5	200.2	753.5	1.39
1×10^{-2}	776.0	178.6	757.6	1.27
5×10^{-2}	982.2	322.2	934.9	1.40
2,4-D esters				
0	42.3	21.5	37.0	1.77
1×10^{-4}	122.0	43.5	114.3	1.49
1×10^{-3}	340.3	136.7	309.9	1.64
1×10^{-2}	808.3	283.9	764.3	1.43

[a] Data for the 2,4-D esters and $CdCl_2$ were based on 9 and 10 observations, respectively. Ethylne was sampled and measured immediately following the completion of the 24-hr incubation period.

(2.44 m × 3.2 mm) and quantified with a gas chromatograph equipped with a flame ionization detector (Tingey *et al.*, 1976; Tingey *et al.*, 1978).

The variances of ethylene measurements were positively correlated with the mean ethylene concentrations (Table I); therefore, the data were transformed to the respective natural logarithms to stabilize the variability (Snedecor and Cochran, 1968). Statistical calculations were performed on transformed data. Dunnett's procedure (Bancroft, 1968), a multiple comparison test which specifically compares treatment means to the control mean, was used to compute a critical value to use in determining significant increases in ethylene production.

3. Results

3.1. STRESS ETHYLENE PRODUCTION

The application of various concentrations of inorganic and organic test compounds to plant rhizospheres increased ethylene production compared to its respective control levels (Figure 2). Different substances induced various types of response. With some compounds (e.g., $CuCl_2$ and 2,4-D) there was a linear increase in stress ethylene

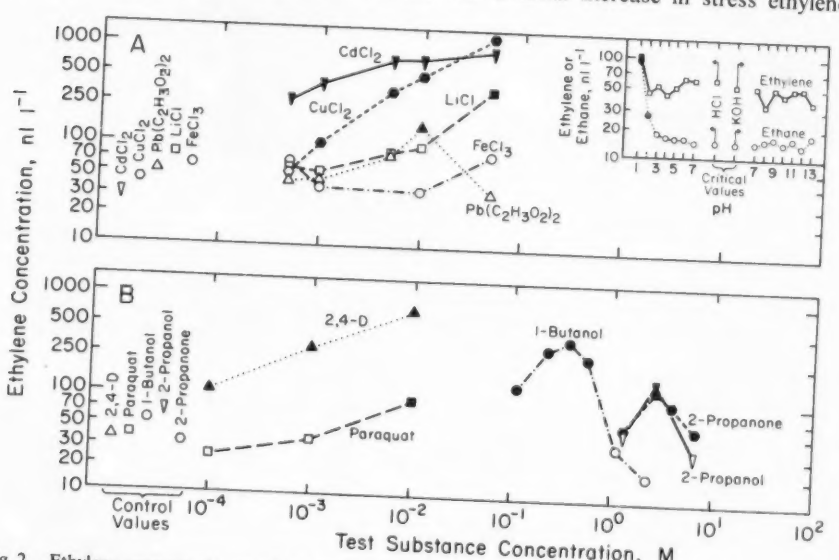

Fig. 2. Ethylene concentration as a function of inorganic test substance concentration (A) and organic test substance concentration (B). A critical value (5% significance level), a statistically significant increase in ethylene, was computed for each test substance. Critical values ($nl\,l^{-1}$) for the test substances were; $CdCl_2$ (45.4); $CuCl_2$ (67.9); $Pb(C_2H_3O_2)_2$ (92.4); LiCl (143.4); $FeCl_3$ (92.0); 2,4-D (59.1); paraquat (68.3); 1-Butanol (86.3); 2-Propanone (48.9); 2-Propanol (116.1). Ethylene and ethane concentrations as a function of pH (A-insert). Solid symbols indicate significant increases in ethylene or ethane above the control. Plants were exposed to light for 2 hr following test substance application. They were then encapsulated and dark-incubated for 24 hr at 26 ± 1 °C. Ethylene and ethane were sampled and measured immediately following the completion of the incubation period.

concentration with increasing test concentration. There was also an increase in stress ethylene production with increasing $CdCl_2$ concentration, but a plateau was reached. With increasing concentrations of other substances (e.g., $Pb(C_2H_3O_2)_2$ and 1-butanol) an increase was followed by a decline in the production of stress ethylene. Ferric chloride did not induce a significant increase in ethylene production over the concentration range.

No single equation adequately described the dose-response curves. Therefore, a critical value (a statistically significant increase in stress ethylene) was computed for each test substance. The critical value for each test substance is shown in the Figure 2 legend. The relative phytotoxicity of each compound was related to the test substance concentration that caused stress ethylene production to exceed its respective critical-value concentration.

At least one concentration of each inorganic compound, except $FeCl_3$, caused the ethylene concentration to exceed its respective critical value (Figure 2A). Cadmium chloride induced significant stress ethylene production at the lowest test substance concentration (5×10^{-4} M) and LiCl at the highest concentration (5×10^{-2} M). Based on analysis of the stress ethylene emissions, the relative phytotoxicity of the inorganic compounds was: $CdCl_2 > CuCl_2 > Pb(C_2H_3O_2)_2 > LiCl$. Ferric chloride was not ranked because it did not induce stress ethylene.

Although stress ethylene was not induced by $FeCl_3$, ethane production was increased. Ethane concentrations increased significantly from the basal level of 23.6 nl l^{-1} to 40.7 nl l^{-1} and 97.2 nl l^{-1} at 1 and 5×10^{-2} M concentrations, respectively.

At least one concentration of each organic test substance induced a significant stress ethylene response (Figure 2B). The 2,4-D esters induced a significant response at the lowest test substance concentration (1×10^{-4} M), while 2-propanol induced a significant response at the highest concentration (2.61 M). The test-substance concentrations that caused stress ethylene production to exceed its critical-value concentrations encompassed a much broader range for the organic test substances than for the inorganic ones. Based on the analysis of stress ethylene production, the relative phytotoxicity of the organic compounds was: 2,4-D esters > paraquat dichloride > 1-butanol > 2-propanone > 2-propanol.

3.2. pH EFFECT ON STRESS ETHYLENE

The effect of hydrogen ion concentrations (applied as HCl or KOH solutions and measured before application) on stress ethylene production was measured to determine if the pH of the test solutions had to be controlled during application. Both stress ethylene and ethane production were nonresponsive to pH over a broad range (Figure 2A insert). Ethylene production exceeded its critical value only at pH 1 while ethane production exceeded its critical value at pH 1 and pH 2.

3.3. EFFECT OF PRE-ENCAPSULATION ON STRESS ETHYLENE

To determine if exposure to light following application of test substances increased stress ethylene production, the influence of 0- or 2-hr light periods prior to encapsulation (pre-encapsulation periods) were compared. Light prior to encapsulation should pro-

mote transpiration, increasing root uptake of test compounds. To assess the influence of pre-encapsulation periods, the ratios of stress ethylene production at the 2- and 0-hr periods were compared (Table II). Ratios greater than 1.00 indicated more stress ethylene was produced following the 2-hr pre-encapsulation period than following the 0-hr period. The 2-hr pre-encapsulation period increased stress ethylene production for the 2,4-D esters, $Pb(C_2H_3O_2)_2$ and $CdCl_2$ and significantly reduced production from Na_2HAsO_4 and paraquat dichloride. However, the pre-encapsulation period had no significant influence on other test substances (e.g., $CuCl_2$ and 1-butanol).

TABLE II

Stress ethylene production as a function of pre-encapsulation time[a]

Test substance	Concentration, (M)	Ethylene production (stressed/control[b])		Pre-encapsulation ratio (2 hr/0 hr)	Standard geometric error
		0-hr Pre-encapsulation	2-hr Pre-encapsulation		
2,4-D esters	0.01	9.2	20.7	2.25	1.29
$Pb(C_2H_3O_2)_2$ 3 H_2O	0.01	1.9	3.0	1.58	1.43
$CdCl_2$ 2.5 H_2O	0.01	16.8	24.0	1.43	1.25
$CuCl_2$ 2 H_2O	0.01	10.8	12.2	1.13	1.33
1-Butanol	0.36	8.1	7.3	0.90	1.23
2-Propanone	2.72	5.3	4.5	0.85	1.41
Paraquat dichloride	0.01	3.5	2.5	0.71	1.15
Na_2HAsO_4 7 H_2O	0.01	10.8	1.4	0.13	1.24

[a] Geometric means for 1-butanol, 2-propanone and paraquat dichloride were based on 6 observations, the 2,4-D esters on 9 observations, $Pb(C_2H_3O_2)_2$, $CdCl_2$, and $CuCl_2$ on 10 observations and Na_2HAsO_4 on 15 observations.

[b] The ethylene increase was computed from the following equation: ethylene (stressed/control) = exp [$\log_e$ mean stress ethylene conc. – $\log_e$ mean control ethylene conc]. Ethylene was sampled and measured immediately following the completion of the 24-hr incubation period. The standard geometric error is for the 2-hr/0-hr pre-encapsulation ratio.

Some test substances did not induce stress ethylene production when a 2-hr pre-encapsulation was used; however, foliar injury occurred. Sodium arsenate induced stress ethylene production only in the absence of a pre-encapsulation period, while $Na_2B_4O_7$ required pre-encapsulation periods longer than 2 hr to induce stress ethylene production. Sodium borate (1×10^{-2} M) did not increase ethylene production following either a 0- or 2-hr pre-encapsulation period; however, extending the pre-encapsulation period to 10 hr increased production to 495.0 nl l^{-1} which exceeded the critical value of 115.1 nl l^{-1}. At 5×10^{2} M $Na_2B_4O_7$, stress ethylene (619.9 nl l^{-1}) was induced following a 5-hr pre-encapsulation period.

4. Discussion

Research with air pollutants indicated that stress ethylene provided an objective and more sensitive measure of stress than assessment of foliar injury (Tingey *et al.*, 1976;

Tingey *et al.*, 1978; Tingey, 1980). Stress ethylene induced by ozone and cadmium was associated with foliar injury and also reduced plant growth (Tingey, 1980). Stress ethylene was induced within 1 to 5 hr by air pollutants (O_3, Cl_2, SO_2) and $CdCl_2$ and persisted for less than 24 hr following a single stress application (Tingey, 1980). This rapid induction of the stress ethylene response indicated that analysis of ethylene emissions provided a faster technique for quantifying plant stress than growth analysis, tissue chemical analysis or foliar injury assessment. Although preliminary results indicated that shorter encapsulation periods (1–3 hr) may be adequate for some compounds (e.g., $CdCl_2$), we recommend a 24-hr encapsulation to increase the probability of detecting stress ethylene production.

A broad range of test substances applied to the rhizosphere induced production of stress ethylene; this is consistent with the concept that the stress ethylene response is a relatively nonspecific stress response (Abeles, 1973; Elstner and Konze, 1976; Tingey, 1980).

We defined the toxicity threshold as the test-substance concentration which caused ethylene production to exceed its critical-value concentration. The test-substance concentration which induced the stress ethylene response and not necessarily the magnitude of the response is the most important issue. Because the threshold concentrations varied among the compounds, the test substances could be ranked according to their relative phytotoxicity (i.e., the more phytotoxic the test substance, the lower the threshold concentration) in a manner analogous to other determinations of toxicity (e.g., LD_{50} or EC_{50} values).

The relative phytotoxicity rankings of the metals from the stress ethylene tests were consistent with the general metal classification and toxicity scheme proposed by Nieboer and Richardson (1980). Although $FeCl_3$ induced stress ethylene in citrus species when applied as a foliar dip or spray (Ben-Yehoshua and Biggs, 1970), no significant response was measured following application to the rhizosphere. Roots absorb iron primarily in the ferrous form and presumably, ferric ion was taken up in insufficient quantities to produce stress-induced ethylene (Chaney *et al.*, 1972).

The application volume (50 ml) was chosen because it wetted the vermiculite and usually produced a few milliliters of leachate. Preliminary data suggested that larger application volumes would increase the sensitivity of the bioasay for $CuCl_2$ but not for $CdCl_2$. Stress ethylene was also produced following the root application of chemicals to hydroponically grown soybeans (Hogsett *et al.*, 1980). The use of a hydroponic system might increase the bioassay sensitivity by reducing the interaction of the test substances with the vermiculite. However, plants from both systems exhibited similar ranges of responses following test substance application, suggesting that the interaction of test substances with vermiculite was not a major problem. Vermiculite was selected because of this observation and also because it was simpler to use.

The lack of a stress ethylene response over a broad pH range in the vermiculite medium suggested that the hydrogen-ion concentration had minimal direct involvement in phytotoxicity. Therefore, a broad range of substances or different chemical species of a single substance might be applied to the rhizosphere for evaluation without concern for the

hydrogen-ion concentration. The significant ethane increase at pH 1 and pH 2 suggested that ethane may serve as a marker for pH effects and probably indicated tissue breakdown (Elstner and Konze, 1976). The use of ethane as an indicator of pH effects was supported in $FeCl_3$ studies. The pH values of the $FeCl_3$ solutions (1 and 5×10^{-2} M) were 2.65 and 2.00, respectively.

The pre-encapsulation period influenced the amount of stress ethylene measured. To detect a stress ethylene response for some compounds, either no pre-encapsulation (Na_2HAsO_4) or periods longer than 2 hr ($Na_2B_4O_7$) were required. The appropriate pre-encapsulation period appears to be test-substance dependent. If no stress ethylene response occurs with a 2-hr encapsulation, additional periods should be used to confirm a negative response.

Membrane stability is important to ethylene production (Lieberman, 1979). False negative responses (i.e., no stress ethylene production induced by phytotoxic chemicals) may occur if the tissue is rapidly disorganized (Elstner and Konze, 1976) or if the compound is an inhibitor of ethylene biosynthesis. The decreased ethylene levels observed with the higher concentrations of $Pb(C_2H_3O_2)_2$, 1-butanol, 2-propanone and 2-propanol may reflect rapid tissue breakdown associated with loss of membrane integrity. The organic solvents caused increased ethylene evolution, a major loss of electrical resistance which was interpreted as increased leakiness of membranes and visible evidence of membrane damage following their injection into cotton bolls (Guinn, 1977). Cobalt ion (applied as $CoCl_2$), an inhibitor of ethylene biosynthesis (Samimy, 1978; Yu and Yang, 1979), failed to induce stress ethylene production following application of a range of concentrations to the rhizosphere even when the pre-encapsulation period was extended through 72 hr (K. D. Rodecap, unpublished results).

Foliar injury asessment is recommended as an adjunct to the measurement of stress ethylene production to detect the phytotoxicity of those substances which produce false negative responses. Although quantitative foliar injury ratings were not made in this study, Na_2HAsO_4, $Na_2B_4O_7$ and $CoCl_2$ and all substances (Figure 2), except $FeCl_3$ and KOH produced foliar injury at the higher concentrations.

In summary, stress ethylene was induced by a wide array of c[illegible]icals applied to the rhizosphere. Analysis of the stress-induced ethylene emissions permitted faster detection of phytotoxic compounds and provided a simpler method to determine their relative phytotoxicities than gowth analysis, tissue chemical analysis or foliar injury determination.

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References

Abeles, F. B.: 1973, *Ethylene in Plant Biology*. Academic Press. New York.
Bancroft, T. A.: 1968, *Topics in Intermediate Statistical Methods*. Vol. I. The Iowa State University Press. Ames, Iowa.

Baur, J. R. and Morgan, P. W.: 1969, 'Effects of Picloram and Ethylene on Leaf Movement in Huisache and Mesquite Seedlings', *Plant Physiol.* **44**, 831–838.

Ben-Yehoshua, S. and Biggs, R. H.: 1970, 'Effects of Iron and Copper Ions in Promotion of Selective Abcission and Ethylene Production by Citrus Fruit and the Inactivation of Indoleacetic Acid', *Plant Physiol.* **45**, 604-607.

Chaney, R., Brown, J. C., and Tiffin, L. O.: 1972, 'Obligatory Reduction of Ferric Chelates in Iron Uptake by Soybeans', *Plant Physiol.* **50**, 208–213.

Elstner, E. F. and Konze, J. R.: 1976, 'Effect of Point Freezing on Ethylene and Ethane Production by Sugar Beet Leaf Disks', *Nature* **263**, 351–352.

Guinn, G.: 1977, 'Effects of Some Organic Solvents on Ethylene Evolution from Young Cotton Bolls', *Plant Physiol.* **60**, 446–448.

Hogsett, W. E., Raba, R. M., and Tingey, D. T.: 1980, 'Inhibition of Stress-Induced Ethylene by the Rhizobitoxine Analog, Aminoethoxy-vinylglycine', *Plant Physiol.* **65**, 84 (supplement).

Lieberman, M.: 1979, 'Biosynthesis and Action of Ethylene', *Annu. Rev. Plant Physiol.* **30**, 533–591.

Maksymowych, R.: 1973, *Analysis of Leaf Development*. Cambridge at the University Press. Cambridge, England.

Nakagaki, Y., Hirai T., and Stahmann, M. A.: 1970, 'Ethylene Production by Detached Leaves Infected with Tobacco-Mosaic Virus', *Virology*. **40**, 1–9.

Nieboer, E. and Richardson, D. H. S.: 1980, 'The Replacement of the Nondescript Term 'Heavy Metals' by a Biologically Significant and Chemically Significant Classification of Metal Ions', *Environ. Pollution (Series B)*. **1**, 3–26.

Samimy, C.: 1978, 'Influence of Cobalt on Soybean Hypocotyl Growth and Its Ethylene Evolution', *Plant Physiol.* **62**, 1005–1006.

Shain, L. and Wheeler, H.: 1975, 'Production of Ethylene by Oats Resistant and Susceptible to Victorin', *Phytopathology*. **65**, 88–89.

Snedecor, G. W. and Cochran, W. G.: 1968, *Statistical Methods*. 6th ed. The Iowa State University Press. Ames, Iowa.

Tingey, D. T.: 1980, 'Stress Ethylene Production – a Measure of Plant Response to Stress', *HortScience*. **15**, 630–633.

Tingey, D. T., Pettit, N., and Bard, L.: 1978, 'Effect of Chlorine on Stress Ethylene Production', *Environ. Expt. Bot.* **18**, 61–66.

Tingey, D. T., Standley, C., and Field, R. W.: 1976, 'Stress Ethylene Evolution: a Measure of Ozone Effects on Plants', *Atmos. Environ.* **10**, 969–974.

Thompson, C. R., Kats, G., Dawson, P., and Doyle, D. A.: 1981, 'Protocol for Measuring the Relative Toxicity of Substances on Plant Foliage', *Bull. Environ. Contam. Toxicol.* **26**, 281–287.

Webb, D. T., Darrow, R. A. and Leather, G. R.: 1975, 'Appraisal of Ethylene Production as a Test for Defoliants', *J. Agric. Food Chem.* **23**, 1113–1115.

Yu, Y-B. and Yang, S. F.: 1979, 'Auxin-Induced Ethylene Production and Its Inhibition by Aminoethoxyvinylglycine and Cobalt Ion', *Plant Physiol.* **64**, 1074–1077.

Yu, Y-B. and Yang, S. F.: 1980, 'Biosynthesis of Wound Ethylene', *Plant Physiol* **66**, 281–285.